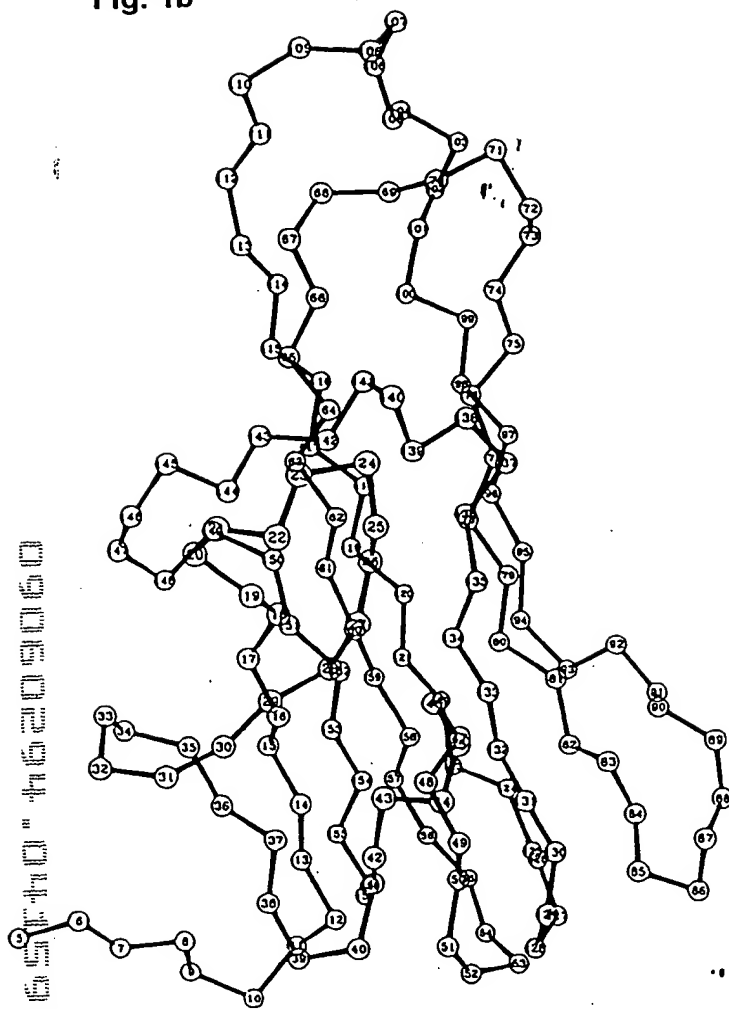


Fig. 1b



Crystal Structure of TNF

Fig. 1a

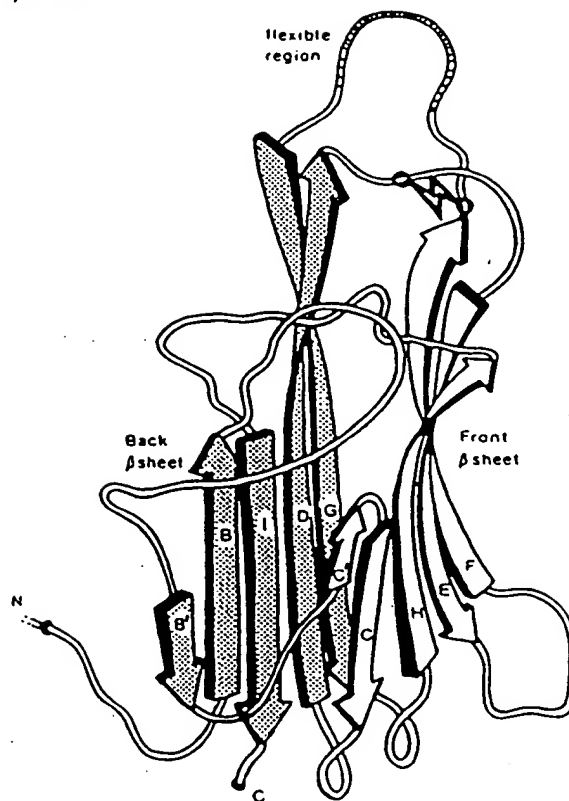


Fig. 1c

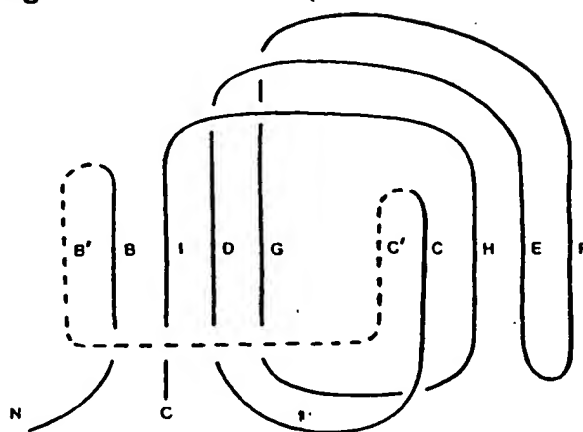


Fig. 2

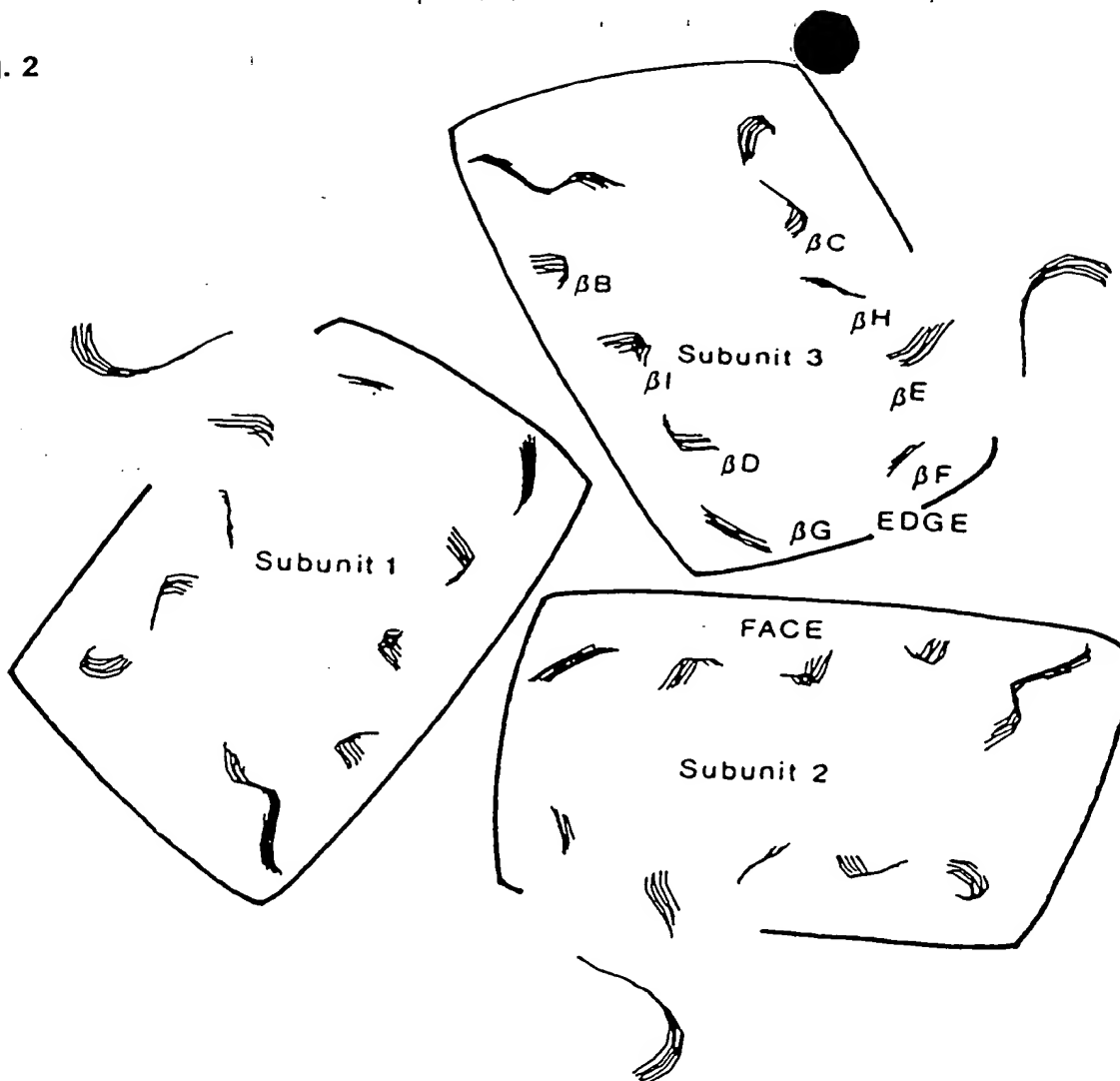


Fig. 3b

Amino acid sequence for human TNFα 8

Accession code: Swissprot P01375

```

-76  MSTESMIRDV ELAEEALPKK TGGPQGSRRCLFLSLFSELI VAGATTLECL
-26  LHFGVIGPQR EEFPRDLSLI SPLAQA
   1  VRSSSRTPSD KPAHVVANP QAEGQLQWLN RRANALLANG VELRDNQLVV
  51  PSEGLYLIYS QVLFKGQGCP STHVLLTHTI SRIAVSYQTK VNLLSAIKSP
 101  CQRETPEGAE AKPWYEPiYL GGVFQLEKGD RLSAEINRPD YLDFAESGQV
 151  YFGIIAL

```

Conflicting sequence F->S at position -14. Disulphide bond between Cys69-101. Signal anchor sequence -41 to -21 (underlined). Myristylation on Lys-58/-57.

Fig. 3a

1 cacaccctga caagctgcca ggcaggttct ctctctctca catactgacc
 51 caeggtccca cctctctctc cctggaaagg acaccatgag cactgaaagc
 101 atgatccggg acgtggagct ggccgaggag gcgctcccca agaagacagg
 151 ggggccccag ggctccaggc ggtgcttggt cctcagcctc ttctccttcc
 201 tgatcgtggc aggcgccacc acgctcttct gcctgctgca ctttggagtg

 251 atcggtcccc agaggggaaga gtcccccagg gacctctctc taatcagccc
 301 tctggcccag gcagtcagat catcttctcg aaccccgagt gacaagcctg
 351 tagcccatgt tgtagcaaac cctcaagctg aggggcagct ccagtggctg
 401 aaccgcccgg ccaatgccct cctggccaat ggcgtggagc tgagagataa
 451 ccagctggtg gtgccatcag agggcctgta cctcatctac tcccagggtc

 501 ttttcaaggg ccaaggetgc cctccacccc atgtgctcct caccacaccc
 551 atcagccgca tcgccgtctc ctaccagacc aaggtcaacc tcctctctgc
 601 catcaagagc ccctgccaga gggagacccc agagggggct gaggccaagc
 651 cctggtatga gcccatctat ctgggagggg tcttccagct ggagaagggt
 701 gaccgactca gcgctgagat caatcgcccc gactatctcg actttgccga

 751 gtctgggcag gtctactttg ggatcattgc cctgtgagga ggacgaacat
 801 ccaaccttcc caaacgcctc ccctgcccc aacctttat taccacctcc
 851 ttcagacacc ctcaacctct tctggctcaa aaagagaatt gggggcttag
 901 ggtcggaacc caagcttaga actttaagca acaagaccac cacttcgaaa
 951 cctgggattc aggaatgtgt ggcctgcaca gtgaagtgtt ggcaaccact

 1001 aagaattcaa actggggcct ccagaactca ctggggccta cagctttgat
 1051 ccctgacatc tggaatctgg agaccagga gcctttggtt ctggccagaa
 1101 tgctgcagga cttgagaaga cctcacctag aaattgacac aagtggacct
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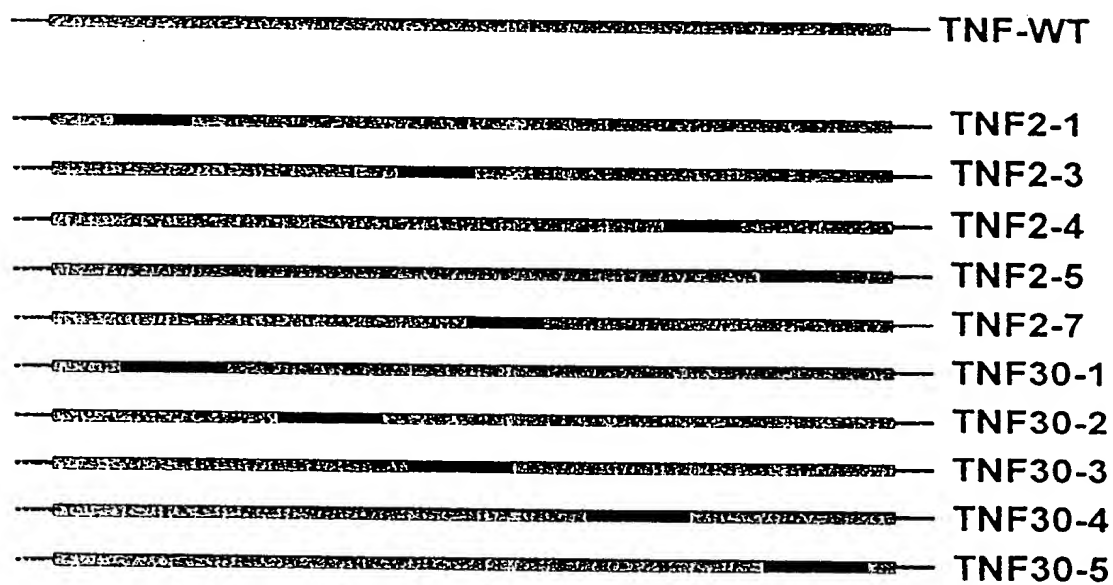
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 1301 tgggagaccg gggtatcctg ggggacccaa tgtaggagct gccttggtc
 1351 agacatgttt tccgtgaaaa cggaggctga acaataggct gtteccatgt
 1401 agccccctgg cctctgtgcc ttcttttgat tatgtttttt aaaatattat
 1451 ctgattaagt tgtctaaaca atgctgattt ggtgaccaac tgtcactcat

 1501 tgetgagggc tctgtctccc agggagttgt gtctgtaatc ggectactat
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Fig. 4a

Location of inserted epitopes



865470" 46209060

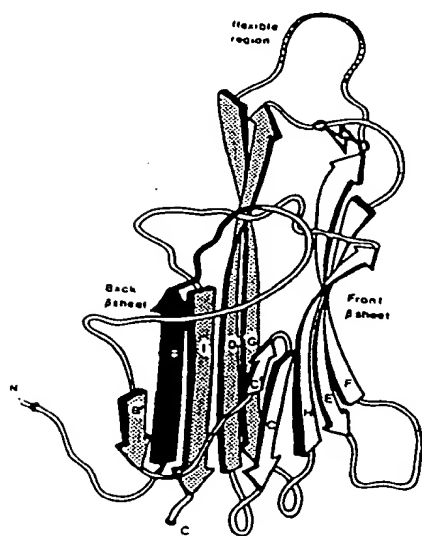
Fig. 4b

[illegible]

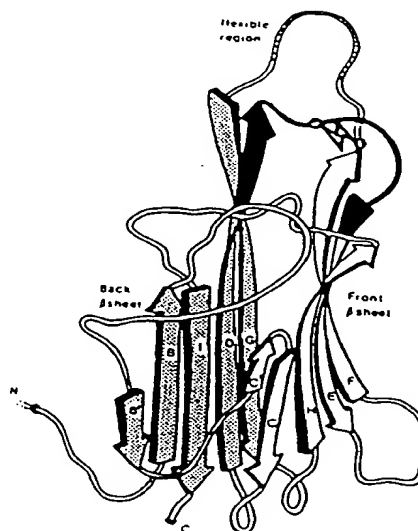
#	81	91	101	111	121	131	141	151
WT	SRIAUSYQTK	VNLLSAIKSP	CQRETPEGAE	AKPWYEPIYL	GGVFQLEKGD	RLSAEINRPD	YLDFAESGQV	YFGIIAL
- 2-1	-----	-----	-----	-----	-----	-----	-----	-----
2-3	-----	-----	-----	-----	-----	-----	-----	-----
2-4	-----	-----	-----	--QYIKANS	KFIGITEL--	-----	-----	-----
2-5	-----	-----	-----	-----	-----	--QYIKANSKF	IGITEL--	-----
- 2-7	NSKFIGITEL	-----	-----	-----	-----	-----	-----	-----
. 30-1	-----	-----	-----	-----	-----	-----	-----	-----
30-2	-----	-----	-----	-----	-----	-----	-----	-----
30-3	SHLE-----	-----	-----	-----	-----	-----	-----	-----
30-4	-----	-----	----FNNFT	VSFWLRVPKV	SASHLE----	-----	-----	-----
- 30-5	-----	-----	-----	-----	-----	---FNNFTVSFW	L RVPKVSASH	LE----

Fig. 5a

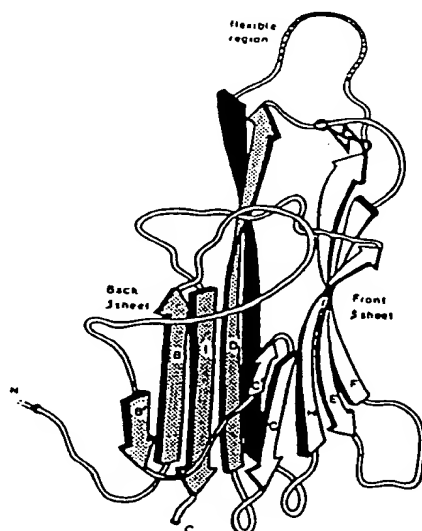
TNF α analogs with the P2 epitope inserted.



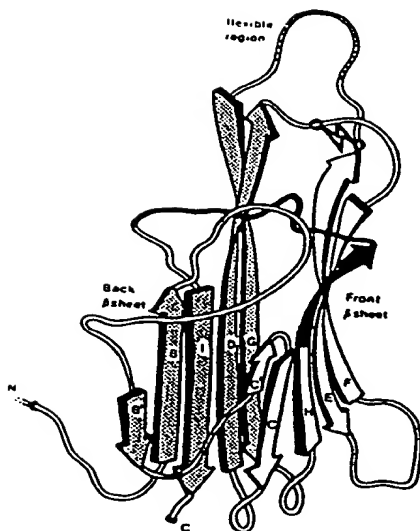
TNF2-1



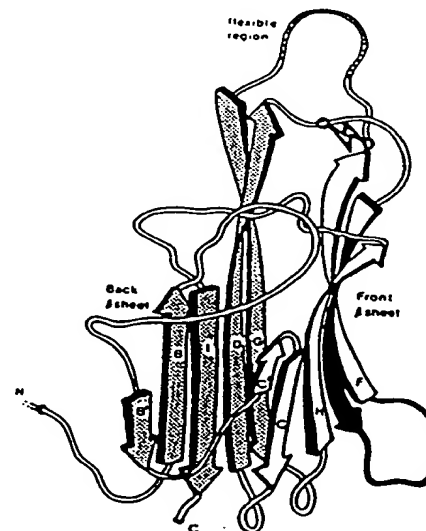
TNF2-3



TNF2-4



TNF2-5

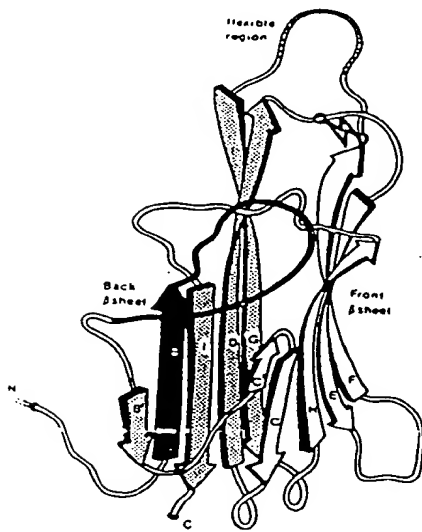


TNF2-7

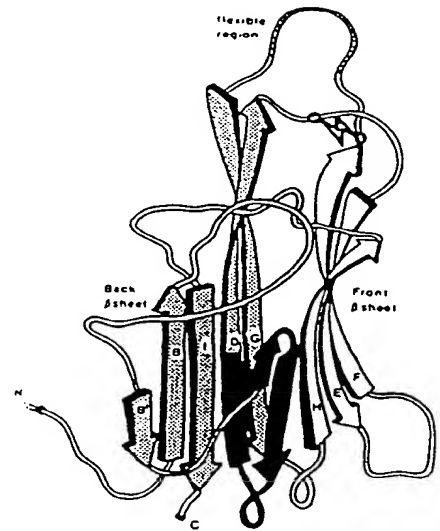
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Fig. 5b

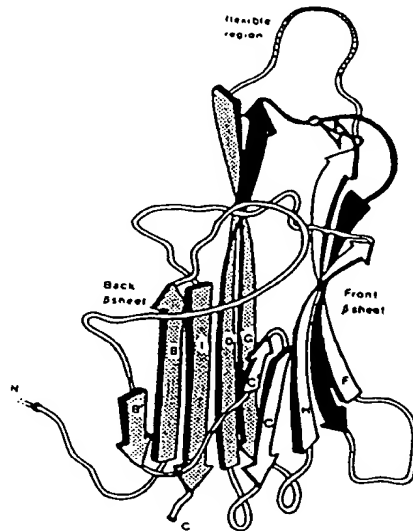
TNF α analogs with the P30 epitope inserted.



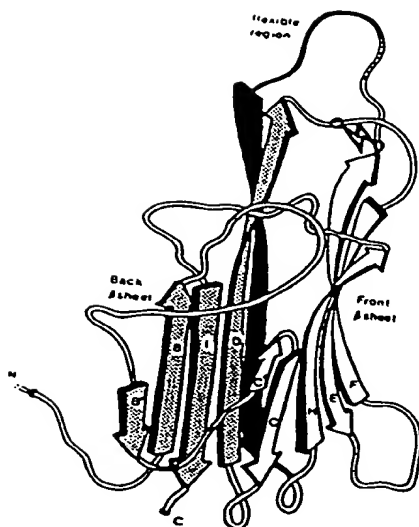
TNF30-1



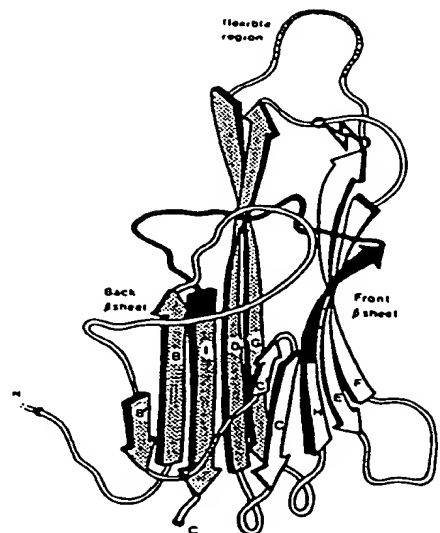
TNF30-2



TNF30-3



TNF30-4



TNF30-5

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Fig. 6

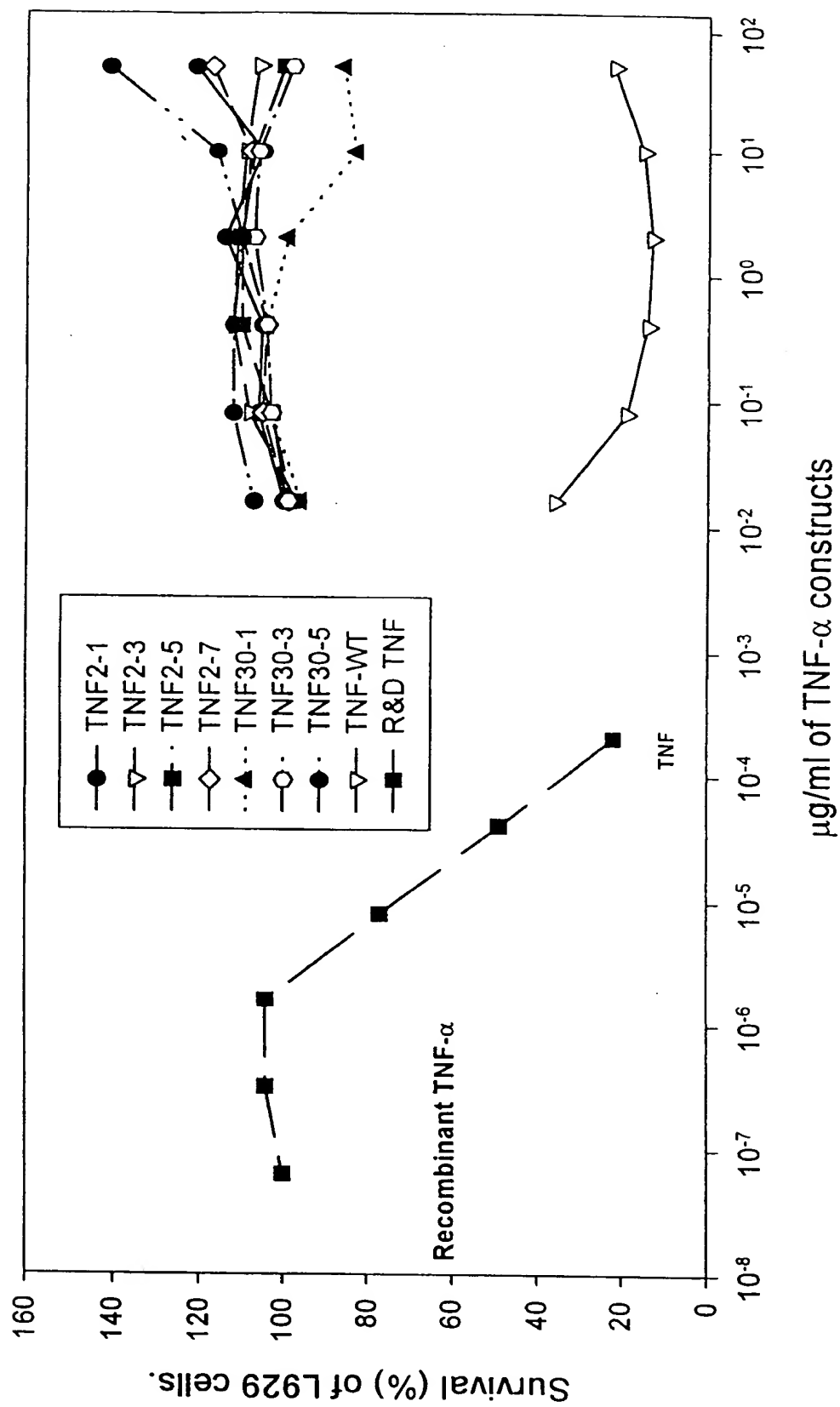


Fig. 7

The anti human TNF α antibody response in rabbits.

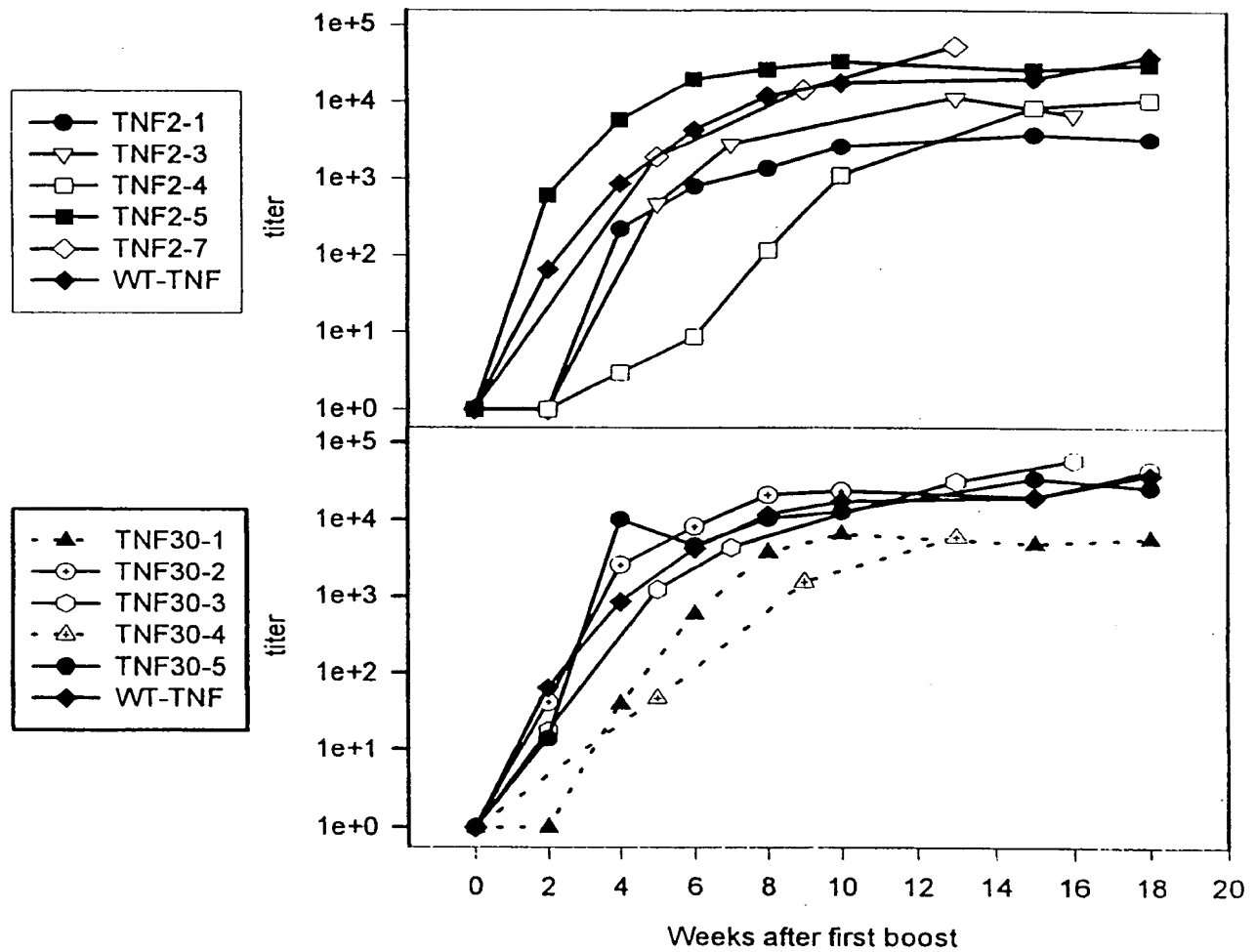


Fig. 8

The ability of P2/P30 modified human TNF α molecules to induce neutralizing antibodies as measured in the L929 cell assay.

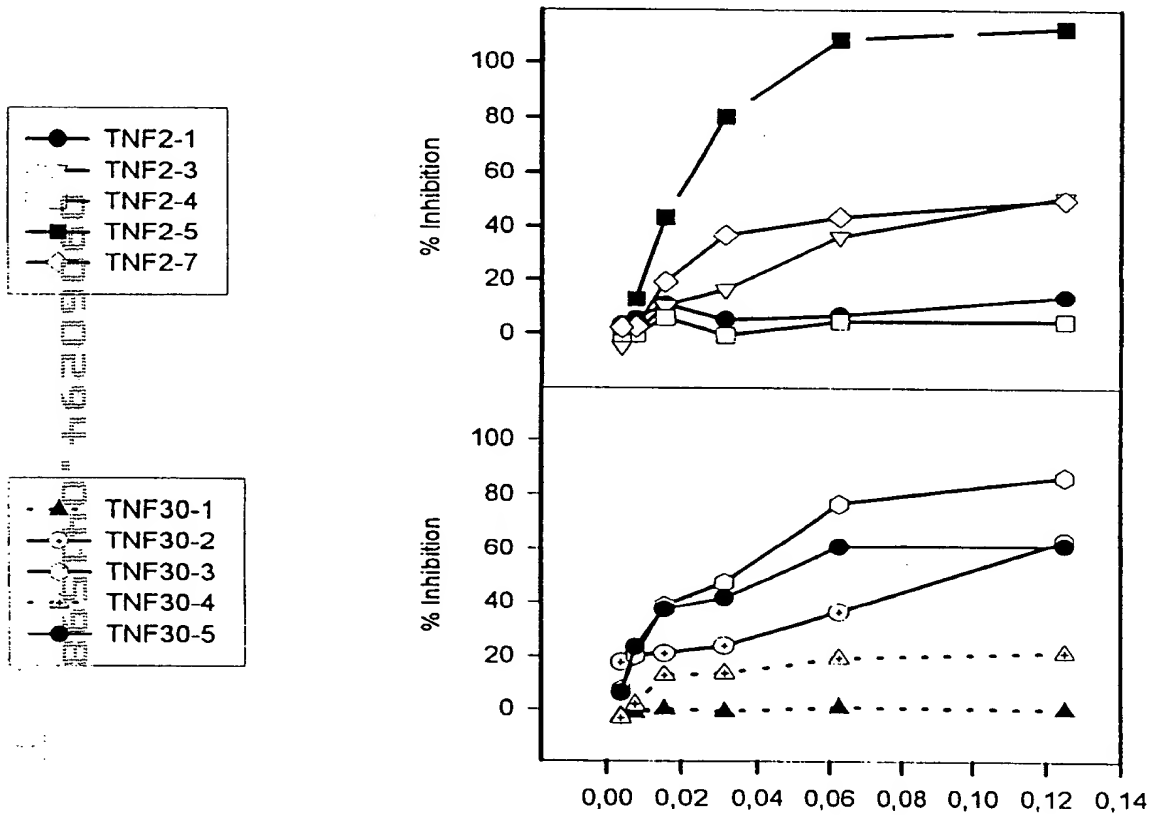


Fig. 9

The ability of P2/P30 modified human TNF α molecules to induce neutralizing antibodies as measured in the receptor assay

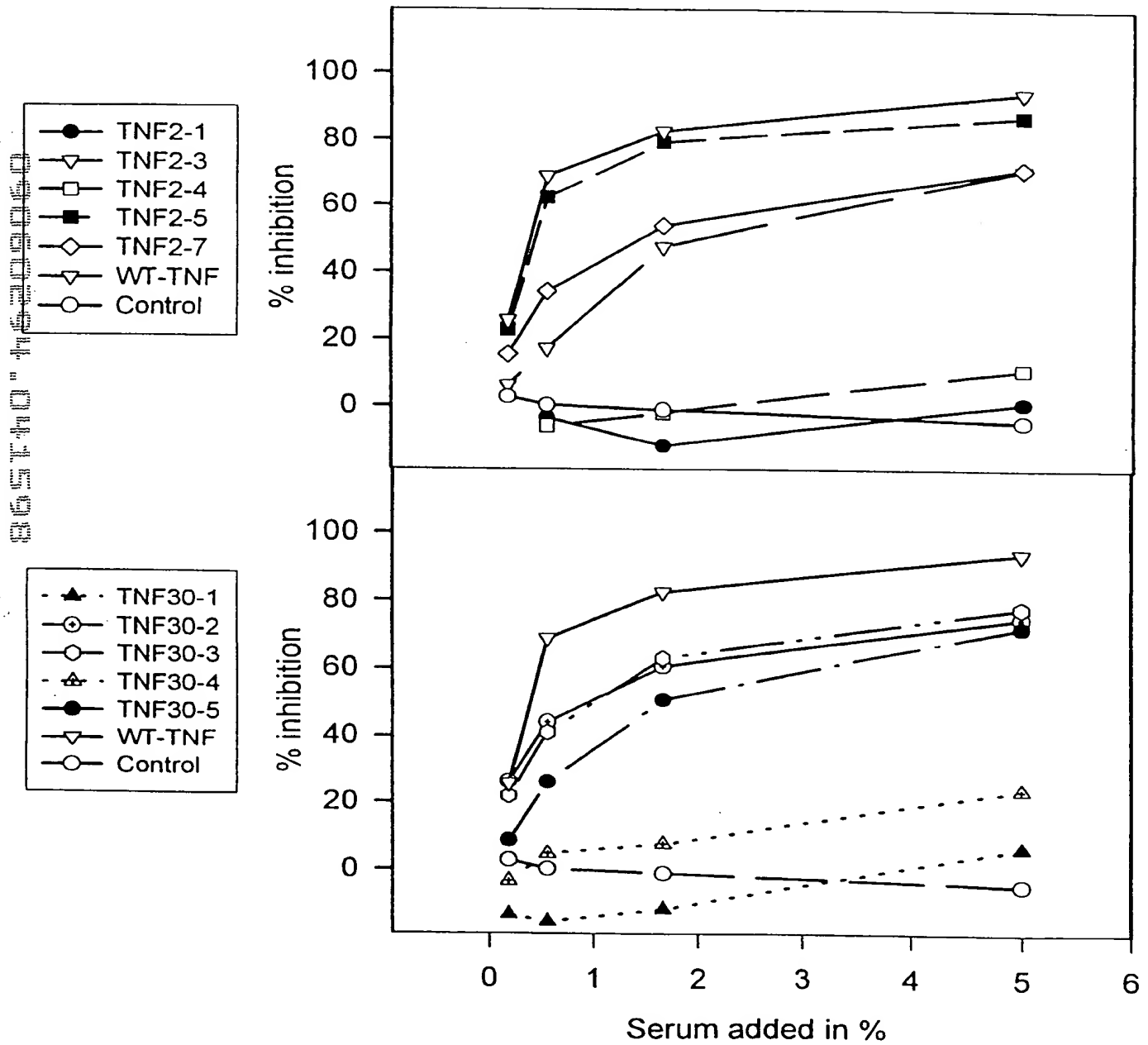


Fig. 10

065T40" 16209060

SR

HB

EC

P2/P30

TT

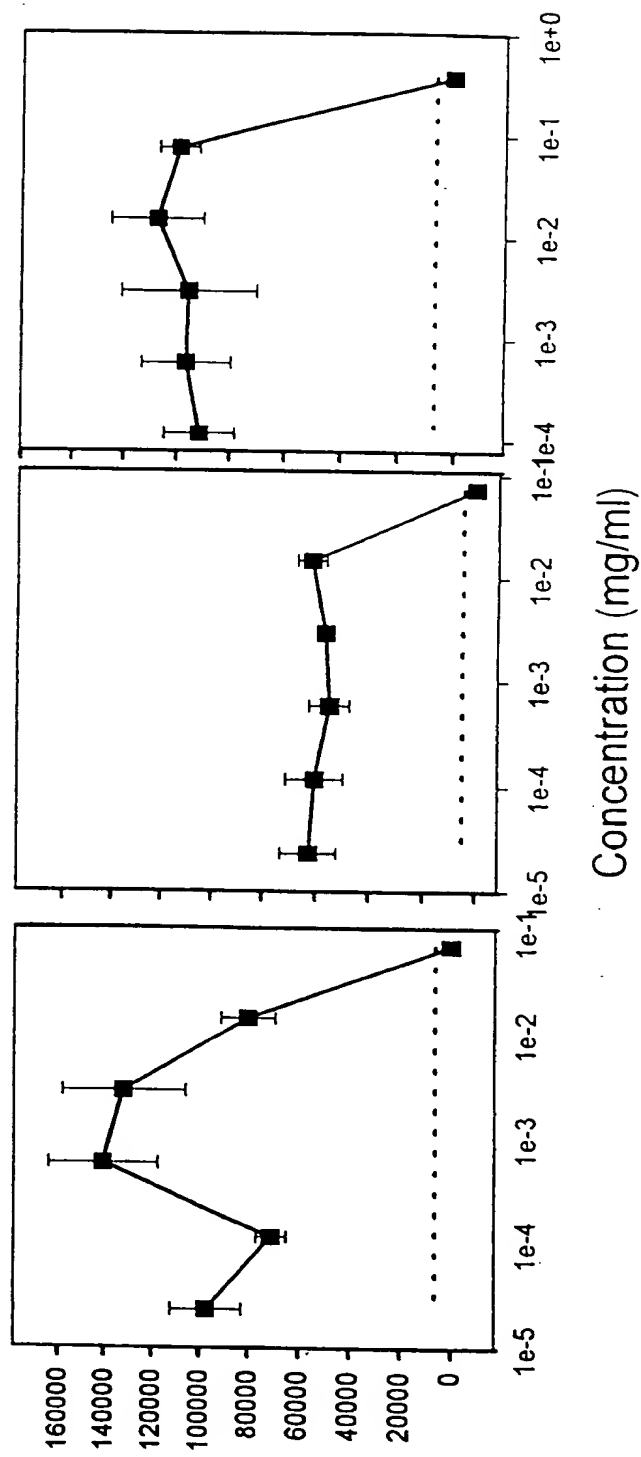
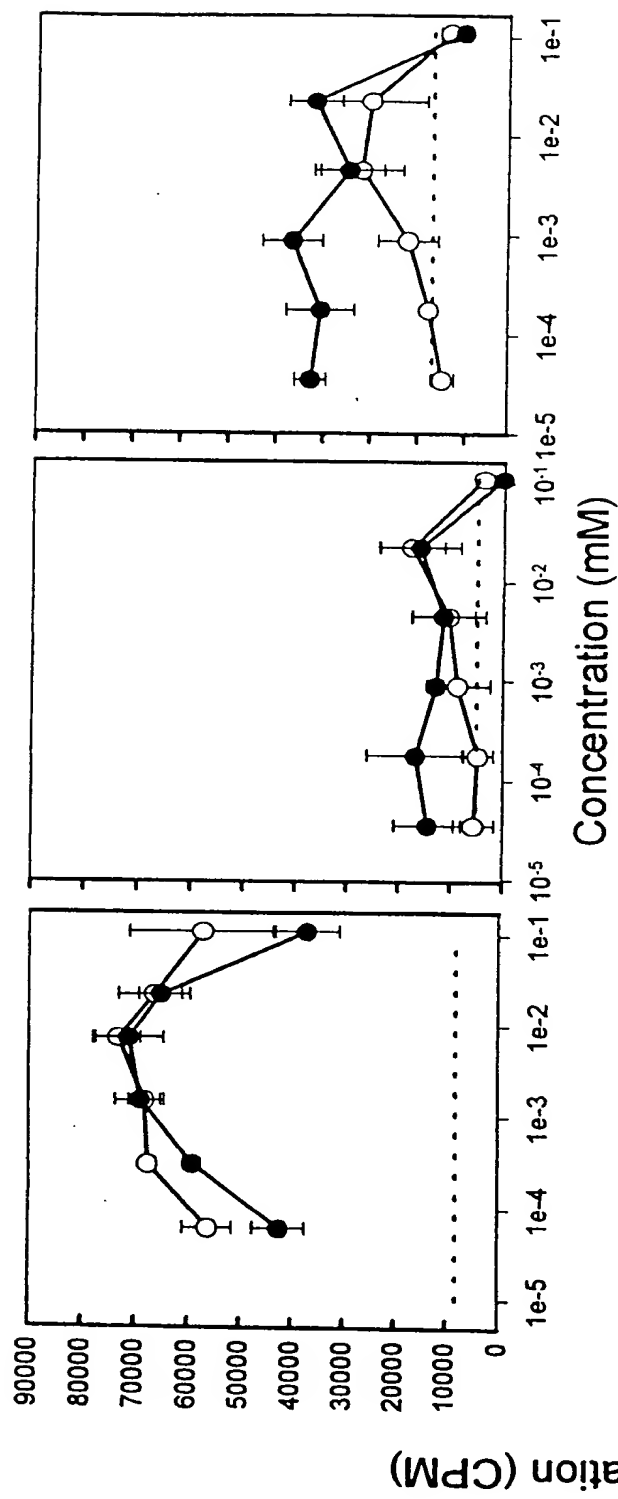


Fig. 12

PBMC Assays

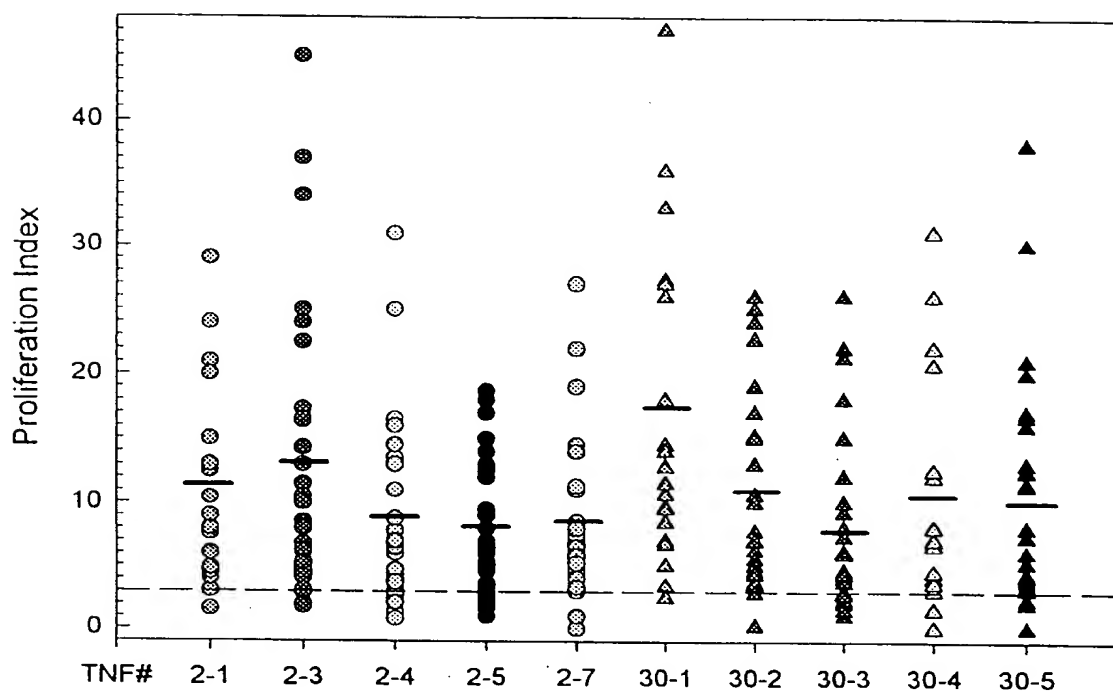


Fig. 13

The PBMC response against P2 and P30 modified TNF α proteins in P2 and P30 specific responders, respectively.

P2/P30 peptides

P2 Proteins

P30 Proteins

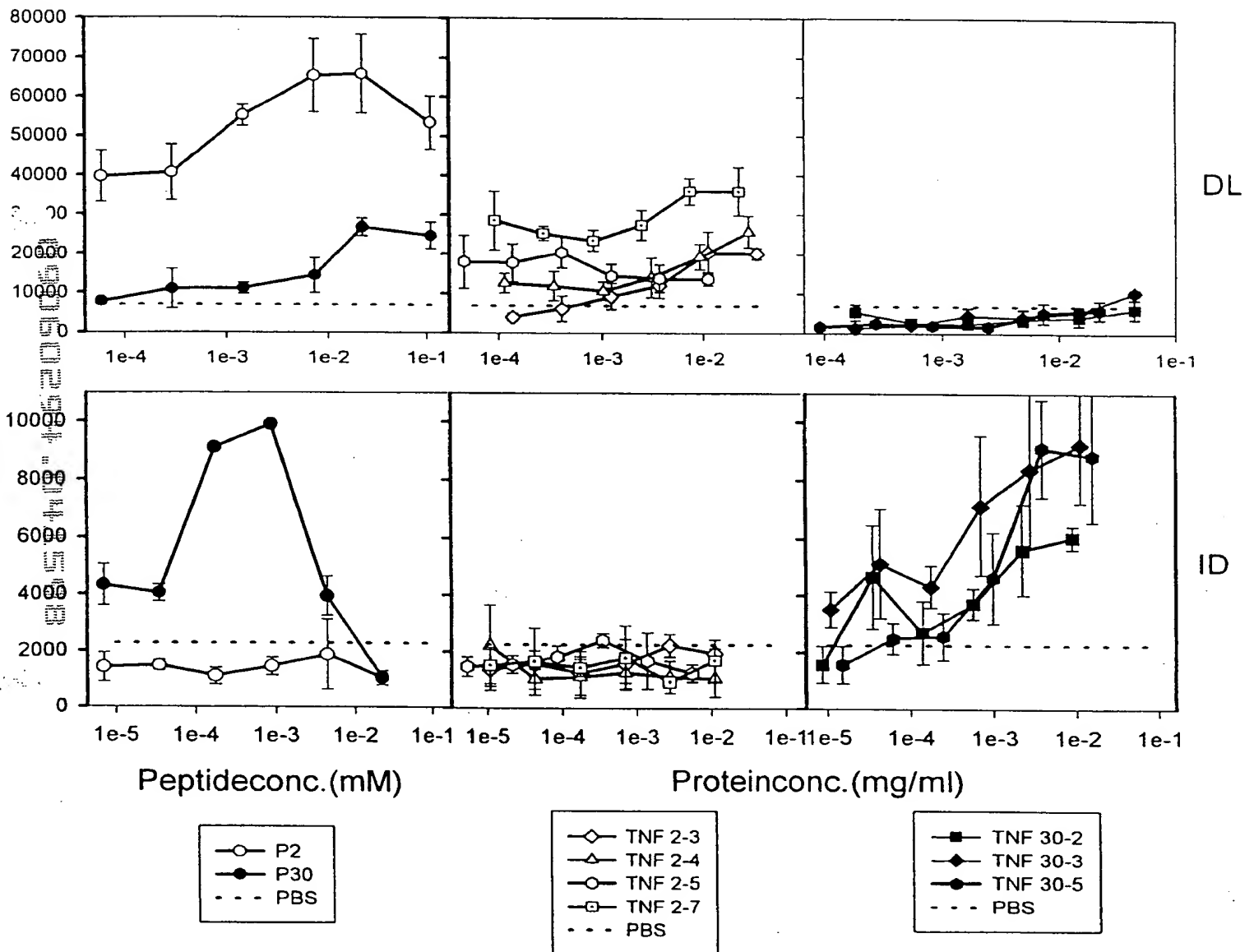


Fig. 14

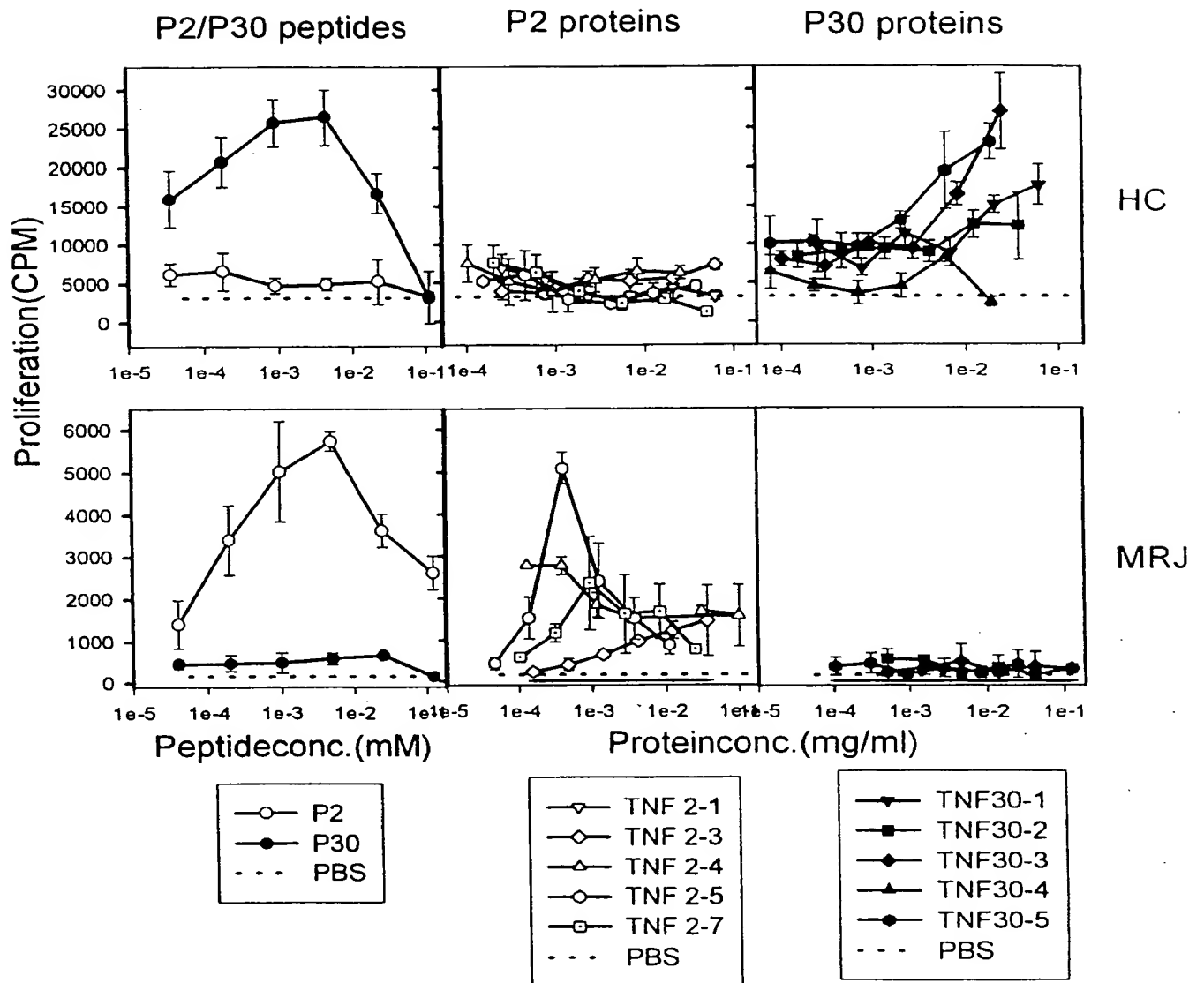
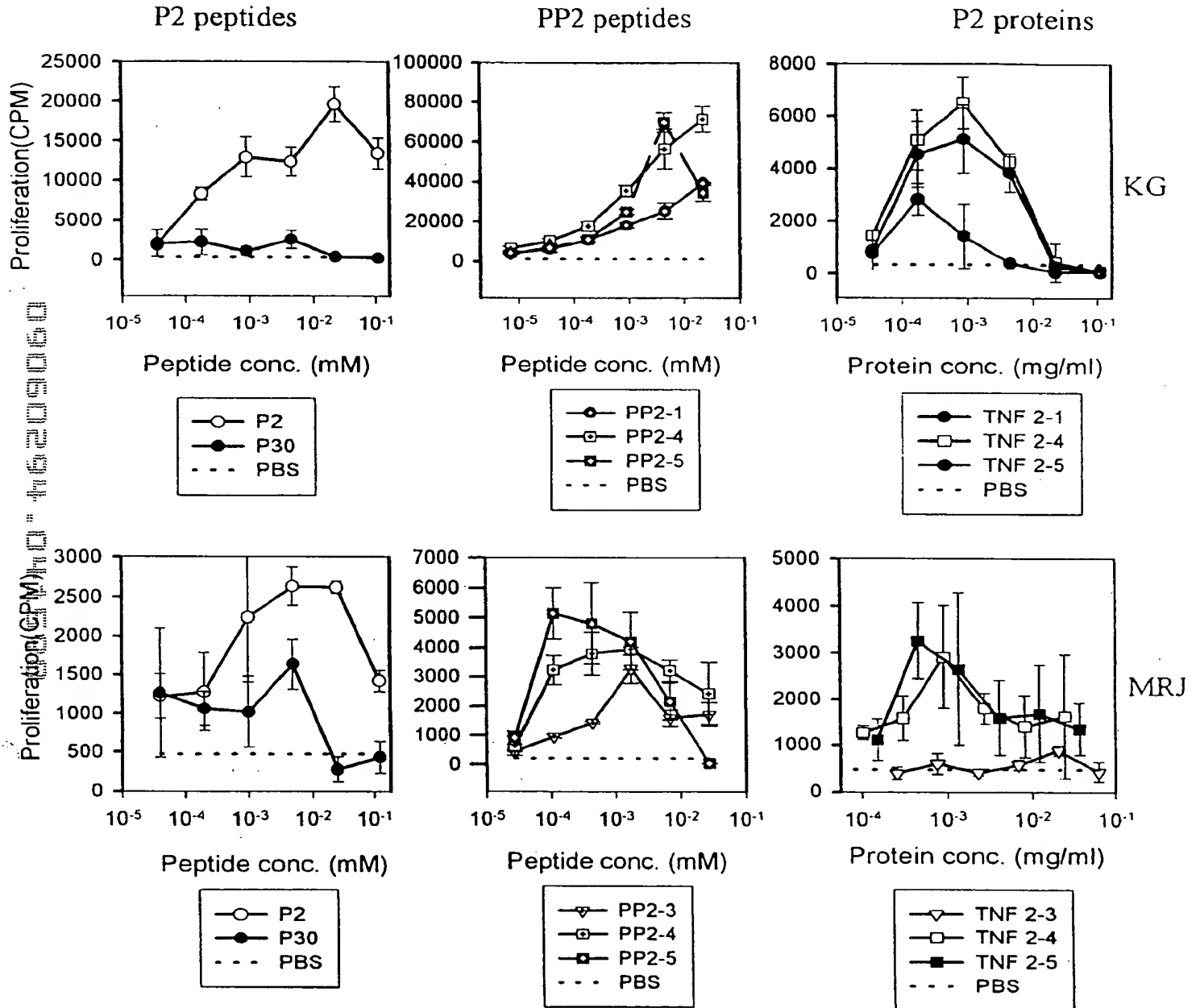
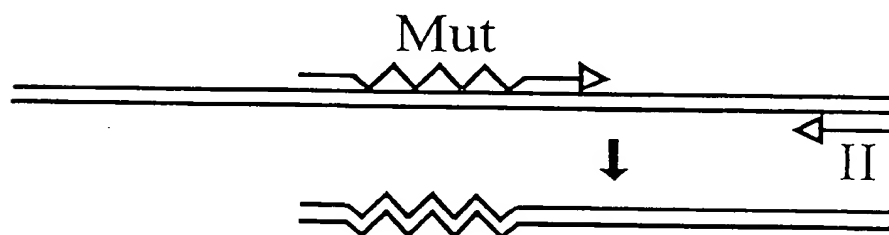
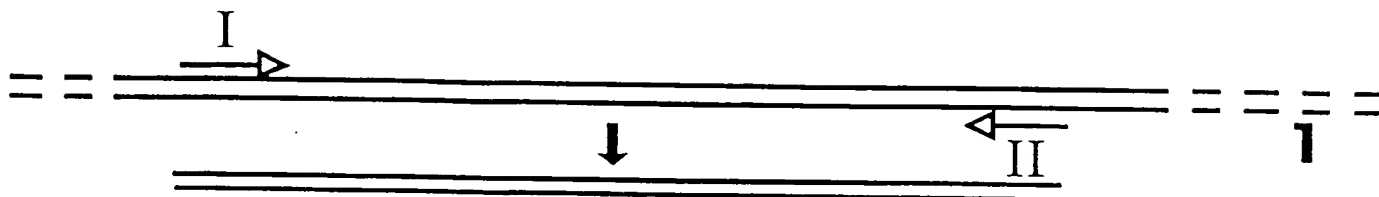


Fig. 15

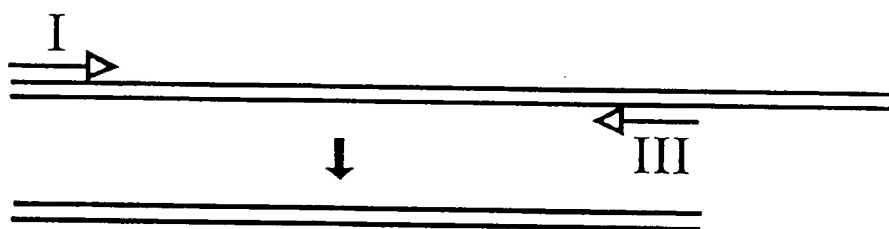
The influence of flanking amino acids on the T cell recognition of P2 and P30



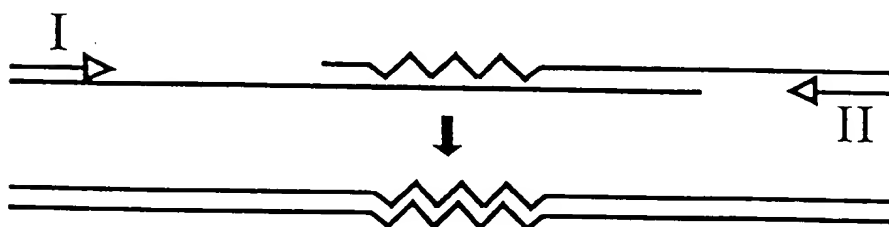
Mutation Strategy



2a



2b



3

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